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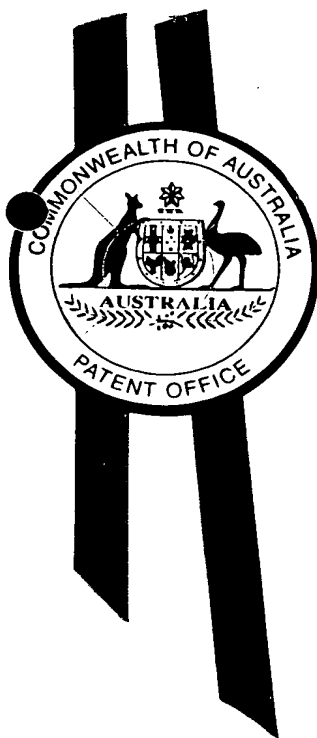
I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES,  
hereby certify that the annexed is a true copy of the Provisional specification in  
connection with Application No. PP 2989 for a patent by THE UNIVERSITY OF  
QUEENSLAND filed on 16 April 1998.

## PRIORITY DOCUMENT

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The University of Queensland

**A U S T R A L I A**

**Patents Act 1990**

**PROVISIONAL SPECIFICATION**

for the invention entitled:

"Novel Peptides"

The invention is described in the following statement:

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example <sup>125</sup>Iodinated MVIIA) are routinely used in pharmacological assays relating to VSCCs.

Whilst the available conotoxins are useful in defining a number of calcium channel sub-types new ligands displaying different binding profiles and affinities may be useful in further  
5 defining channel sub-types.

In addition to their use as research tools,  $\omega$ -conotoxins which target N-type calcium channels have been proposed for use in the treatment of a variety of conditions including ischaemia induced brain injury, acute psychotic episodes which may be drug induced or result from a  
10 psychiatric disorder, diseases which cause bronchoconstriction, and chronic pain. One particular  $\omega$ -conotoxin, known as MVIIA or, in its synthetic form, SNX-111, is in clinical trials for some of these applications.

Despite these advances in the use of  $\omega$ -conotoxins the presently available compounds are not  
15 ideal therapeutics. For example, SNX-111 has been reported to cause hypotension as a result of action at peripheral channels. Another of the  $\omega$ -conotoxins, GVIA, is a potent antagonist of N-type calcium channels but binds to such channels in an irreversible manner, and accordingly is unsuitable as a therapeutic. Many other of the known  $\omega$ -conotoxins do not have an adequate level of selectivity for the N-type channel to be deemed suitable therapeutic  
20 candidates; blockade of P/Q-type channels may lead to death.

Accordingly there exists a need for new therapeutic agents which have a selectivity for N-type calcium channels over P/Q type channels, and which may be useful in the treatment of conditions related to N-type calcium channels.

25

In a first aspect of the present invention there is provided an isolated, synthetic or recombinant  $\omega$ -conotoxin peptide in which the fourth loop between cysteine residues 5 and 6 comprises the following sequence of amino acids:

30

SGTVGR [SEQ ID NO:1]

- 3 -

or such a sequence which has undergone one or more amino acid substitutions, or side chain modifications.

Preferably the fourth loop consists of the above sequence, or such a sequence which has  
5 undergone one or more conservative amino acid substitutions or side chain modifications.

The first, second and third loops of the  $\omega$ -conotoxin peptide correspond to the loops of a naturally occurring  $\omega$ -conotoxin peptide, or such a sequence of amino acids which has undergone one or more amino acid substitutions, additions or deletions.

10

Substitutions encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an amino acid residue contained in a polypeptide is replaced with another naturally-occurring amino acid of similar character, for  
15 example Gly→Ala, Val→Ile→Leu, Asp→Glu, Lys→Arg, Asn→Gln or Phe→Trp→Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a polypeptide is substituted with an amino acid having different properties, such as naturally-occurring amino acid from a different group (eg.  
20 substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

25

Additions encompass the addition of one or more naturally occurring or non-conventional amino acid residues. Deletion encompasses the deletion of one or more amino acid residues.

Preferably, amino acid substitutions are conservative.

30

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As stated above the present invention includes peptides in which one or more of the amino acids has undergone sidechain modifications. Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with  $\text{NaBH}_4$ ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with  $\text{NaBH}_4$ .

10 The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

15

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides.

25 Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

30

Proline residue may be modified by, for example, hydroxylation in the 4-position.

- 5 -

A list of some amino acids having modified side chains and other unnatural amino acids are shown in Table 1.

TABLE 1

5				
	Non-conventional amino acid	Code	Non-conventional amino acid	Code
	$\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
10	$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
	aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
	carboxylate		L-N-methylaspartic acid	Nmasp
	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbornyl-	Norb	L-N-methylglutamine	Nmgln
15	carboxylate		L-N-methylglutamic acid	Nmglu
	cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
20	D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
25	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
30	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg

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	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
5	D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgab
	D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchexa
	D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpen
	D- $\alpha$ -methylasparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
	D- $\alpha$ -methylaspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
10	D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- $\alpha$ -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
	D- $\alpha$ -methylleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
15	D- $\alpha$ -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
20	D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- $\alpha$ -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
	D- $\alpha$ -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
25	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
30	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr

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	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
5	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
10	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl- $\alpha$ -naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	$\gamma$ -aminobutyric acid	Gabu	N-( <i>p</i> -hydroxyphenyl)glycine	Nhtyr
15	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
	L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methylassparagine	Masn
	L- $\alpha$ -methylasspartate	Masp	L- $\alpha$ -methyl- <i>t</i> -butylglycine	Mtbug
20	L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- $\alpha$ -methylglutamine	Mgln	L- $\alpha$ -methylglutamate	Mglu
	L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomophenylalanine	Mhphe
	L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- $\alpha$ -methylleucine	Mleu	L- $\alpha$ -methyllysine	Mlys
25	L- $\alpha$ -methylmethionine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
	L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
	L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
	L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
	L- $\alpha$ -methyltryptophan	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr
30	L- $\alpha$ -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
	N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe



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carbamylmethyl)glycine  
 1-carboxy-1-(2,2-diphenyl- Nmbe  
 ethylamino)cyclopropane

carbamylmethyl)glycine

5

These types of modifications may be important to stabilise the peptide if administered to an individual or for use as a diagnostic reagent.

Other derivatives contemplated by the present invention include a range of glycosylation  
 10 variants from a completely unglycosylated molecule to a modified glycosylated molecule.  
 Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

The  $\omega$ -conotoxins of the present invention are typically amidated at the C-terminal however  
 15 compounds with a free carboxyl terminus or other modifications at the C-terminal are  
 considered to be within the scope of the present invention. Preferably the peptides are  
 amidated or have a free carboxyl.

Preferably the peptide has a second loop which comprises a sequence selected from:

20

SKLMYD [SEQ ID NO: 2].

SRLMYD [SEQ ID NO: 3], and

DRLMYD [SEQ ID NO: 4],

25 or such a sequence which has undergone one or more conservative amino acid substitution  
 or side chain modifications.

In a particularly preferred embodiment the  $\omega$ -conotoxin peptide has the following sequence:

30 CVID

CKSKGAKCSKLMYDCCSGSCSGTVGRC [SEQ ID NO: 5]

1

2

3

4

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The four loops are shown underlined. This peptide was isolated from *Conus catus* and has been designated herein as CVID. The peptide has been shown to have a high potency and a high selectivity for N-type calcium channel over P/Q-type calcium channel in receptor binding assays. Two modified forms of CVID have also been shown to have a high potency  
 5 and high selectivity for N-type calcium channel. These are designated R<sup>10</sup>-CVID and D<sup>9</sup>R<sup>10</sup>-CVID as follows:

R <sup>10</sup> -CVID	CKSKGAKCSRLMYDCCSGSCSGTVGRC [SEQ ID NO: 6]
10 D <sup>9</sup> R <sup>10</sup> -CVID	CKSKGAKCDRLMYDCCSGSCSGTVGRC [SEQ ID NO: 7]

In another embodiment of the present invention there is provided a chimeric  $\omega$ -conotoxin peptide in which one or more of loops 1 to 3 of conotoxin CVID have been substituted with the corresponding loop of a different  $\omega$ -conotoxin.

15

Some known conotoxins are as follows:

MVIIA (SNX-III)	CKGKGAKCSRLMYDCCTGSCRSGKC [SEQ ID NO: 8]
20 MVIIC	CKGKGAPCRKTMYDCCSGSCGRRGKC [SEQ ID NO: 9]
GVIA	CKSOGSSCSOTSYNCCRSCNOYTKRCY [SEQ ID NO: 10]

In the sequence for GVIA the "O" refers to 4-hydroxy proline (Hyp). This amino acid  
 25 residue results from post translational modification of the encoded peptide and is not directly encoded by the nucleotide sequence.

Chimeric  $\omega$ -conotoxins contemplated by the present invention include DADD, DAGD and GGGD. Where a D, A or a G represent loops selected from CVID, MVIIA or GVIA  
 30 respectively. Accordingly DADD corresponds to loops 1, 3 and 4 being selected from

- 10 -

CVID and loop 2 being selected from MVIIA, this chimeric  $\omega$ -conotoxin is the same as R<sup>10</sup>-CVID.

The peptides according to the present invention preferably have a selectivity for N-type  
5 calcium channel over P/Q type calcium channel. Those skilled in the art would be able to readily determine the selectivity of the peptides for these calcium channels using standard techniques.

Iodinated GVIA and MVIIC are high affinity ligands for the N-type and P/Q type  
10 calcium channel receptors respectively and are routinely used in receptor binding assays (Kristipati *et al.*, 1994; Nadasdi *et al.*, 1995). Such assays may be used to test the calcium channel binding activity of the peptides of the present invention. Functional assays such as those described by Lew *et al.* (1997) may also be useful in determining activity at N-type calcium channels.

15

The  $\omega$ -conotoxins of the present invention may be prepared using standard peptide synthetic methods followed by oxidative disulfide bond formation. For example, the linear peptides may be synthesised by solid phase methodology using BOC chemistry, as described by Schnoltzer *et al* (1992). Following deprotection and cleavage from the  
20 solid support the reduced peptides are purified using preparative chromatography. The purified reduced peptides are oxidised in buffered systems, for example as described in example 2. The oxidised peptides were purified using preparative chromatography.

References describing the synthesis of conotoxins include Sato *et al*, Lew *et al* and WO  
25 91/07980.

The  $\omega$ -conotoxins may also be prepared using recombinant DNA technology. A nucleotide sequence encoding the desired peptide sequence may be inserted into a suitable vector and protein expressed in an appropriate expression system. In some instances,  
30 further chemical modification of the expressed peptide may be appropriate, for example

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C-terminal amidation.

Naturally occurring CVID was isolated from *Conus catus* by assay guided fractionation of the venom followed by sequencing of the purified peptide.

5

The invention further provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to sequence encoding a  $\omega$ -conotoxin peptide as described above.

10 In a further aspect of the present invention there is provided a nucleic acid probe comprising a sequence of nucleotides encoding or complementary to a sequence encoding  $\omega$ -conotoxin peptides having a fourth loop of CVID, said probe encoding or complementary to all or part of loop 4 of the  $\omega$ -conotoxin CVID, or such a sequence which has undergone one or more amino acid substitution or side chain modifications.

15

In a particularly preferred embodiment the nucleic acid probe comprises a sequence of nucleotides encoding or complementary to a sequence encoding the sequence shown in SEQ ID NO: 1.

20 As used herein a reference to a "probe" includes reference to a primer used in amplification or a probe for use in direct hybridization.

Still another aspect of the present invention is directed to antibodies to the  $\omega$ -conotoxin peptides according to the invention. Such antibodies may be monoclonal or polyclonal and  
25 may be selected from naturally occurring antibodies to the peptides or may be specifically raised to the peptides. In the case of the latter, the peptides may first need to be associated with a carrier molecule. The antibodies of the present invention are particularly useful as therapeutic or diagnostic agents.

30 In this regard, specific antibodies can be used to screen for the peptides according to the invention. Techniques for such assays are well known in the art and include, for example,

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sandwich assays and ELISA. Knowledge of peptide levels may be important for monitoring certain therapeutic protocols.

The nucleic acid molecules of the present invention may be DNA or RNA. When the  
5 nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic  
10 molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli*, *Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

15 Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and a gene capable of encoding a peptide according to the invention.

20 Preferably, the gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of the gene portion in an appropriate cell.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic  
25 cells comprising same.

In view of their high potency and selectivity towards N-type calcium channel over P/Q type the  $\omega$ -conotoxin peptides of the present invention may be useful in the reduction of neuronal damage following ischemia, production of analgesia, enhancement of opiate  
30 analgesia, treatment of schizophrenia or the treatment of stimulant psychoses.

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Accordingly in a further aspect of the present invention there is provided a composition comprising: an isolated or recombinant  $\omega$ -conotoxin peptide in which the fourth loop between cysteine residues 5 and 6 comprises the following sequence of amino acids:

5 SGTVGR [SEQ ID NO: 1]

or such a sequence which has undergone one or more conservative amino acid substitutions, and

10 a pharmaceutically acceptable carrier or diluent.

Preferably the composition is in the form of a pharmaceutical composition.

There is also provided the use of an isolated or recombinant  $\omega$ -conotoxin peptide in  
15 which the fourth loop between cysteine residues 5 and 6 comprises the following sequence of amino acids:

SGTVGR [SEQ ID NO: 1]

20 or such a sequence which has undergone one or more conservative amino acid substitutions or side chain modifications in the manufacture of a medicament for the reduction of neuronal damage following ischemia, production of analgesia, enhancement of opiate analgesia, treatment of schizophrenia or the treatment of stimulant psychoses.

25 The invention further provides a method for reducing neuronal damage following ischemia, for the production of analgesia, for enhancement of opiate analgesia, for the treatment of schizophrenia or the treatment of stimulant psychoses including the step of administering to a mammal an effective amount of an isolated or recombinant  $\omega$ -conotoxin peptide in which the fourth loop between cysteine residues 5 and 6 comprises  
30 the following sequence of amino acids:

## SGTVGR [SEQ ID NO: 1]

or such a sequence which has undergone one or more conservative amino acid substitutions or side chain modifications.

5

Preferably the mammal is in need of such treatment, although the peptide may be administered in a prophylactic sense.

As will be readily appreciated by those skilled in the art, the route of administration and  
10 the nature of the pharmaceutically acceptable carrier will depend on the nature of the condition and the mammal to be treated. It is believed that the choice of a particular carrier or delivery system, and route of administration could be readily determined by a person skilled in the art. In the preparation of any formulation containing the peptide  
actives care should be taken to ensure that the activity of the peptide is not destroyed in  
15 the process and that the peptide is able to reach its site of action without being destroyed. In some circumstances it may be necessary to protect the peptide by means known in the art, such as, for example, micro encapsulation. Similarly the route of administration chosen should be such that the peptide reaches its site of action.

20 The pharmaceutical forms suitable for injectable use include sterile injectable solutions or dispersions, and sterile powders for the extemporaneous preparation of sterile injectable solutions. They should be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms such as bacteria or fungi . The solvent or dispersion medium for the injectable solution or dispersion may contain any  
25 of the conventional solvent or carrier systems for peptide actives, and may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of  
30 surfactants. The prevention of the action of microorganisms can be brought about where necessary by the inclusion of various antibacterial and antifungal agents, for example,

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parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include agents to adjust osmolality, for example, sugars or sodium chloride. Preferably, the formulation for injection will be isotonic with blood. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents  
5 delaying absorption, for example, aluminum monostearate and gelatin. Pharmaceutical forms suitable for injectable use may be delivered by any appropriate route including intravenous, intramuscular, intracerebral, intrathecal injection or infusion.

Sterile injectable solutions are prepared by incorporating the active compounds in the  
10 required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred  
15 methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for  
20 example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.  
25 Such compositions and preparations preferably contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained.

30

The tablets, troches, pills, capsules and the like may also contain the components as listed



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- hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.
- 15 The present invention also extends to any other forms suitable for administration, for example topical application such as creams, lotions and gels, or compositions suitable for inhalation or intranasal delivery, for example solutions or dry powders.

Parenteral dosage forms are preferred, including those suitable for intravenous, intrathecal, or intracerebral delivery.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated;

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each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular  
5 therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in  
10 effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 µg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 µg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by  
15 reference to the usual dose and manner of administration of the said ingredients.

In order to facilitate an understanding of the invention reference will be made to the examples and figure which illustrate some preferred embodiments of the invention. However it is to be understood the generality of the preceding description is not to be  
20 superseded by the particularity of the following description.

Referring to the figure:

Figure 1 is an example of a nucleic acid sequence encoding CVID. The amino acid  
25 sequence including the leader sequence and terminal glycine is also shown. The nucleic acid sequence and amino acid sequence are also shown as SEQ ID NO: 12 and SEQ ID NO: 13 respectively. The primary nucleotide sequence is 382bp in length and comprises the leader sequence (amino acid residues 1 to 45), the mature peptide (amino acid residues 46 to 73 and boxed), the 3' untranslated region (depicted by lower case lettering immediately  
30 following the region of the mature peptide), and a small portion of the 5' untranslated region that was incorporated into the CSRD-301A primer (depicted in bold italicised lower case

- 18 -

lettering at the start of the sequence). The start and stop codons delineating the peptide coding region are underlined. The putative amino acid sequences for the leader and mature peptides have been translated from the primary nucleotide sequence and are shown in single letter abbreviation below the nucleotide sequence. The numbering above the nucleotide  
5 sequence relates to the position of the amino acid residues taken from the start codon. The position of the CSRD-301A PCR primer within the CVID sequence is highlighted in bold and italicised: the ANCHOR primer would be positioned immediately 3' to the poly-A tail (at 382+bp). An arrow at the arginine residue at position 45 indicates the most probable site for enzymatic cleavage of the leader peptide from the mature peptide.

10

The terminal glycine of the predicted expressed protein is removed by some form of post translational modification to leave an amidated C-terminal cystine in the protein isolated from snails.

15

## EXAMPLES

### Example 1

20 Assay guided fractionation of the venom of *Conus catus* was performed as follows:

The omega conotoxin CVID was isolated originally from the crude venom extracted from the venom ducts of *Conus catus* collected from the Great Barrier Reef, Australia. Using gradient reverse phase HPLC the crude venom was separated into a number of fractions and  
25 these fractions then assayed in a <sup>125</sup>I GVIA binding assay (see Example 4). Fractions active in the binding assay were further purified by assay-guided reverse phase HPLC and the primary structure obtained unambiguously by Edman sequencing. The fraction corresponding to CVID had a retention time of around 25-27 minutes.

30 The reverse phase HPLC was conducted on a Waters 600 HPLC system on preparative and analytical Vydac C18 columns. Samples were typically run using a 1% gradient (100% A, 5

- 19 -

min; 100% A to 60% B, 60 min) at 1 ml/min and monitored at 214 nm. Additional fractionation was at times achieved using size-exclusion HPLC. Fractions for assay were collected either at 1 minute intervals or to correspond to peaks detected with a u.v. detector. The buffer system used for all analysis was A=0.1%TFA in H<sub>2</sub>O and  
 5 B=0.09%TFA, 10%H<sub>2</sub>O, 90%CH<sub>3</sub>CN.

## **Example 2**

The synthesis of peptides was performed according to the following procedures.

10

### **Materials and Methods**

#### *Materials*

15 Synthesis of C-terminal amidated peptides was conducted on *p*-MBHA resin, obtained from Peninsula Laboratories and Peptide Institute, substitution values ranged from 0.66 to 0.93meq/g. C-terminal acids were synthesised on Boc protected PAM resins obtained from Applied Biosystems. Boc protected amino acids were obtained from Peptide Institute, BA Chem, Nova Biochem, Fluka, Biosearch and Auspep. The side-chain protection chosen for  
 20 the boc amino acids was Arg(Tos), Asn(Xan), Asp(OcHex), His(DNP), Lys(ClZ), Thr(Bzl), Tyr(BrZ), Glu(OcHex), Ser(Bzl), HyP(Bzl), Trp(CHO), Cys(*p*-MeBzl), Gln(Xan). All other Boc amino acids used were side-chain unprotected. DMF, DCM, DIEA, TFA, dicyclohexylcarbodiimide and hydroxybenzotriazole were all peptide synthesis grade from Auspep (Melbourne, Australia). Acetonitrile and methanol (Hipersolve-Far UV grade) were  
 25 from BDH (Poole, UK). Water was obtained from a tandem Millipore Milli-RO - Milli-Q system. *p*-cresol and *p*-thiocresol were from Fluka (Germany). HF was supplied by BOC Gases (Brisbane, Australia). Ammonium acetate (AR) and ammonium sulphate (AR) were from AJAX Chemicals (Australia). Guanidine. HCl (99%+) and reduced and oxidised Glutathione were from Sigma Aldrich (USA).

30

#### *Methods*

- 20 -

### Synthesis

Automatic peptide synthesis was conducted on an Applied Biosystems 430A synthesiser, using symmetric anhydride or active ester chemistries to couple Boc-protected amino acids to the resin. Manual stepwise synthesis was conducted using BOC chemistry methodology, where 2mmol of each amino acid is activated using 4ml of 0.5M HBTU in DMF and 470 $\mu$ l DIEA, and *in-situ* coupling takes on average 10min to obtain >99% coupling by quantitative ninhydrin<sup>2</sup> analysis. Both methods involved starting from *p*-MeBHA or PAM resin (0.5mmol scale). Where -OCH<sub>2</sub>-PAM resin was used the first amino acid was on the resin. Removal of the Boc protecting group prior to coupling was accomplished by vortexing or shaking in 100%TFA. DMF was used for flow washes and as the coupling solvent. Each residue (2mmol) was routinely double coupled on the synthesiser and in the manual synthesis when ninhydrin values indicated less than 99% coupling. If coupling remained less than 99%, the remaining amino groups were acetylated using acetic anhydride in DMF (87 $\mu$ l/ml).

### Deprotection and Cleavage

For peptides containing histidine-DNP, the fully protected peptide was first subjected to thiolysis (20%  $\beta$ -mercaptoethanol, 10% DIEA in DMF, 2 x 30min), to remove the side-chain protection. The N- $\alpha$ -Boc group was then removed (TFA, 2 x 1min), and for peptides containing tryptophan-CHO, deformylation was performed using a solution of ethanolamine (1.5g) in 25ml 5% water in DMF (2 x 30min). The peptide was washed with DCM and dried under nitrogen. Cleavage from the resin and simultaneous deprotection of side-chains was carried out in liquefied HF in the presence of the scavengers *p*-cresol and *p*-thiocresol (18:1:1 by volume) at -5 - 5°C for 1.5hr. HF was removed under vacuum, the peptide was precipitated with cold ether, collected by filtration on a sintered funnel and washed with cold ether to remove scavenger adducts. The peptide was dissolved in either 50% AcOH or 45% aqueous acetonitrile, diluted with water and lyophilised.

### 30 Folding and Oxidation

Purified reduced peptides were oxidised at a concentration of 0.02 to 0.05mM in either aqueous 0.33M  $\text{NH}_4\text{OAc}$  / 0.5M  $\text{GnHCl}$ , or aqueous 2M  $(\text{NH}_4)_2\text{SO}_4$  / 0.1M  $\text{NH}_4\text{OAc}$  with pH adjusted to 7.5 - 8.0 using 0.01M  $\text{NH}_4\text{OH}$ . The solution was stirred for 3 to 5 days at 4°C, in the presence of reduced and oxidised glutathione (molar ratio of peptide:GSH:GSSG was 1:100:10 ). The reaction mixtures were sampled periodically and analysed by RP-HPLC and eluant fractions were collected for electrospray mass spectrometric analysis. When LC and MS confirmed that oxidation was complete, the oxidation was terminated by lowering the pH to 2-3 with TFA.

#### 10 Chromatographic Analysis and Purification

A Waters 600 HPLC system equipped with an auto-injector was used for all RP-HPLC. Analytical RP-HPLC was conducted on a Waters Delta pak C18, 300A (0.39 x 30cm) column or a Vydac C18, 5 $\mu$  (0.46 x 25cm) column. Samples were run using a 1% gradient (100%A, 5min; 100%A to 60%B, 60min), at 1ml/min and monitored at 214nm.

The buffer system used for all analysis was A=0.1%TFA in  $\text{H}_2\text{O}$  and B=0.09%TFA, 10% $\text{H}_2\text{O}$ , 90% $\text{CH}_3\text{CN}$ .

A Vydac C18, 5 $\mu$  (1.0 x 25cm) column was used for semipreparative RP-HPLC and a Vydac C18, 10 $\mu$  (2.2 x 25cm) column was used for preparative RP-HPLC.

The crude reduced peptides were purified by preparative chromatography, using a 1% gradient (100%A to 80%B, 80min) with a flow rate of 8ml/min and u.v detection at 230nm. Fractions were collected and analysed by electrospray mass spectrometry. Fractions which gave the desired mass were then analysed by analytical RP-HPLC to confirm purity, and those fractions which were pure were combined and lyophilised to give the reduced peptide. Oxidised peptides were purified by loading the acidified reaction mixtures onto a preparative column at a flow rate of 8ml/min, washing through with 100%A until all oxidation buffer had eluted and then applying a 1% gradient (100%A to 80%B, 80min) with a flow rate of 8ml/min and u.v detection at 230nm. Fractions were collected and analysed as for the reduced peptides. If further purification was necessary the peptide was repurified on a semipreparative column on a 1% gradient (100%A to 80%B, 80min) with a flow rate of

- 22 -

3ml/min and u.v detection at 230nm. Fractions were collected and analysed as before.

### Mass Spectrometry

5 Mass spectra were measured on a PE Sciex API-III triple quadrupole Ion Spray mass spectrometer. Data was obtained in the positive ion mode by the accumulation of data in the range 400-2100 amu from several scans using a scan step of 0.1 amu, and a delay time of 0.3 s.

Peptides were dissolved at a concentration of 1mg/ml in 45% aqueous acetonitrile  
10 containing 0.1% TFA. HPLC fractions were used without further treatment. Samples were delivered to the orifice via a glass capillary by direct injection (5-20ul) using a Rheodyne injector into a 30-40ul/min solvent flow of 50% aqueous acetonitrile containing 0.05% TFA. The resultant data was subjected to deconvolution (Hypermass - MacSpec 3.2, SCIEX, Canada) to determine the Mr of the observed protonated species.

15

Other high-resolution data were obtained on a Bruker Spectrospin BioAPEX external-ion-source Fourier transform electrospray mass spectrometer at a magnetic field of 4.7 T.

Data for some of the peptides synthesised is tabulated below

20

**Table 1** List of synthesised peptides, optimal yields and Mass values.

PEPTIDE	REDUCED YIELD %	OXIDISED YIELD %	EXPECTED MASS (Mr,Da)	OBSERVED MASS (Mr,Da)
CVID	36	35	2755	2755
R <sup>10</sup> - CVID	36	40	2784	2784
25 D <sup>9</sup> R <sup>10</sup> -CVID	33	29	2812	2812

### Example 3

30 Isolation and Characterisation of the CVID Gene Sequence

## RNA extraction and cDNA synthesis

Two specimens of *Comus catus* were collected from Lady Elliot Island on the Queensland  
 5 Great Barrier Reef. The animals were anaesthetised on ice, and dissected to remove the  
 venom duct in a region from the venom bulb to the proboscis. The ducts were sectioned,  
 placed in a buffer containing guanidinium thiocyanate/N-lauroyl sarcosine, then emulsified  
 with manual grinding. Poly-A tailed mRNA was extracted from the mixtures using the  
 Pharmacia Biotech QuickPrep mRNA purification system.

10

Strand-1 cDNA was 3' end synthesised from the *C. catus* poly-A mRNA templates using a  
 NotI-d(T)<sub>18</sub> bifunctional primer (5'-AACTGGAAGAATTCGCGGCCGCAGGAAT<sub>(18)</sub>-3')  
 (Pharmacia Biotech) in conjunction with Superscript II reverse transcriptase (Gibco BRL).  
 The resultant cDNA templates were used to manufacture double stranded cDNA using a  
 15 RNaseH/DNA polymerase procedure as per the Pharmacia Biotech cDNA Timesaver  
 protocol. Marathon (Clontech) adaptors were then added to the 5' and 3' ends of the ds-  
 cDNA molecules to complete the cDNA construction. A representation of a complete  
 coneshell venom peptide cDNA molecule is shown in figure 1.

20

## PCR derivation of CVID and related cDNA sequences

PCR was carried out on samples containing ds-cDNA from *C. catus*, the CSRD-301A  
 primer (5' - ATCATCAAAATGAAACTGACGTC - 3'), the ANCHOR primer (5' -  
 25 AACTGGAAGAATTCGCGGCCGCAGGAAT - 3') and an appropriate *Tag* polymerase  
 (Biotech International) and buffer (25mM Mg, 100uM deoxy-nucleotides, buffered at pH  
 8.5) in a thermal cycler (Omnigene) at 95°C/2 mins for 1 cycle, 95°C/30 sec - 55°C/60 sec -  
 72°C/90 sec for 35 cycles, and 72°C/10 mins for 1 cycle. This PCR produced a  
 heterogeneous DNA product of approximately 380 bp to 500bp. Sequence analysis of  
 30 clones derived from this PCR product have shown it to contain the sequence CVID as well  
 as other related venom peptide sequences.



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### Cloning and sequencing of CVID

The DNA product produced from the CSRD-301A-ANCHOR driven PCR of *C. catus* cDNA was electrophoresed in low melting point agarose and excised. The DNA was  
5 extracted from the agarose on Qiagen columns, rephosphorylated with T4 DNA kinase (Progen), blunt ended with Klenow polymerase (Progen), and ligated with T4 DNA ligase (Progen) into the multiple cloning site of dephosphorylated *Sma-I* cut pUC-18 plasmid vector DNA (Pharmacia Biotech). The vector DNA was electrotransformed into Bluescript *E.coli* cells, to produce a library of clones representing the PCR product. Aliquots of the  
10 library were plated onto LB<sub>amp</sub> plates, and individual clones selected and propagated overnight in TB<sub>amp</sub> broth. Plasmid DNA was purified from the culture using the RPM system (BIO-101), and the PCR DNA inserts within the vector sequenced using the pUC-18 forward and reverse primers (Pharmacia Biotech), di-deoxy terminator sequencing chemistries (Perkin Elmer) on ABI 373 sequencers. The sequence data was analysed using  
15 Sequence Navigator software (Applied Biosystems).

### CVID gene sequence

20 Clone number Cca-6 within the *C. catus* CSRD-301A/ANCHOR venom duct cDNA PCR library provided the sequence for the CVID peptide. The nucleotide sequence and the anticipated translation of the associated amino acid sequence for the CVID gene are shown in figure 1. Further analysis of this clone library revealed a total of eight clones with identical sequence. All clones were sequenced in both orientations to produce a consensus  
25 sequence.

The CVID sequence has the following characteristics:

- A coding sequence of 222 base pairs which translate to 73 amino acids
- A predicted mature peptide sequence of 28 amino acids. It should be noted that in

- 25 -

the protein isolated from snails the terminal glycine of the predicted expressed protein is removed by some form of post translational modification to leave an amidated C-terminal cystine.

- 5 • The predicted mature peptide has a six cysteine framework in the pattern of;  
C-a<sub>6</sub>-C-a<sub>6</sub>-CC-a<sub>3</sub>- C-a<sub>6</sub>-C

The nucleotide and amino acid sequence of both the leader peptide and the mature peptide are not identical to any known peptide sequence.

10

#### Example 4

##### **Radioligand binding assays**

##### 15 *Preparation of <sup>125</sup>I-GVIA and <sup>125</sup>I-MVIIC*

Peptides were iodinated using IODO-GEN® (Fraker P.J. *et al.*, 1978) (1,3,4,6-tetrachloro-2a,6a-diphenyl-glycuoluril) [Ahmad S.N. *et al.*, 1988, Cruz L.J. *et al.*, 1986]. 10 ml of the peptide, 5 ml (5.75 mg/ml, 17.4 mCi/mg) Na<sup>125</sup>I (DuPont NEN®, New research products,  
20 Boston) and 25 ml of sodium phosphate buffer (50 mM, pH 7.4) were added to an eppendorf tube coated with IODO-GEN® (Pierce, Rockford, USA) and incubated for 5 mins. The reaction mixture was vortexed and transferred to an eppendorf tube containing 10 ml of the peptide of interest. This mixture was then allowed to react for another 5 min prior to purification by HPLC.

25

Preparative HPLC of <sup>125</sup>I-labelled peptides was performed on a Waters 680 gradient controller equipped with two Waters 510 HPLC pumps and a Waters 481 absorbance detector. Peptides were analysed on Vydac reverse phase C-18 analytical column (4.6 x 250 mm) eluted at 1 ml/min with a linear gradient of 0-67% of solvent B over 100 min: solvent  
30 A, 1% TFA (trifluoroacetic acid); solvent B, 90% ACN + 0.09% TFA. Separation was monitored at 214 nm and 1 ml fractions were collected. Fractions of interest were detected

with a LKB Wallac 1272 automatic Gamma counter.

To confirm the identity of iodinated peptides, mass spectrometry was performed on a PE-SCIEX API III mass spectrometer (PESciex, Thornhill, Ontario, Canada). HPLC fractions  
5 from peptides iodinated with non-radioactive  $K^{127}I$  were injected directly into the mass spectrometer. Mass spectra were acquired on a Apple Macintosh IIfx computer using the software package MacSpec (Sciex, Toronto).

#### *Rat Membrane Preparation*

10

Rat membrane was prepared according to the procedure of Wagner *et al.* (1988). Rats were sacrificed by cervical dislocation and their brains removed and immediately frozen in liquid nitrogen. Frozen brains were stored at  $-78^{\circ}C$  until required. Three brains (wet weight , 6.25 g) were thawed and homogenised with ultraturrex (IKA, 170 Watt) in 125 ml 50 mM  
15 HEPES pH 7.4. Homogenised brain was centrifuged at 16000rpm (35000g) for 20min at  $4^{\circ}C$  and the supernatant discarded. The pellet was resuspended by further homogenisation in 50mM HEPES, pH 7.4, 10 mM EDTA and incubated at  $4^{\circ}C$  for 30 min. Centrifugation was repeated as above and the supernatant discarded. The pellet was resuspended in 125ml 50mM HEPES, pH 7.4 (1:20 dilution) and stored at  $-78^{\circ}C$ .

20

#### *Binding Assays.*

Binding experiments were performed as previously described (Kristipati *et al.*, 1994, Nadasdi *et al.*, 1995). Ligand binding assays were run in triplicate in glass tubes at room  
25 temperature. Briefly, assays were performed in 12 x 75 mm borasilicate culture tubes at room temperature and incubated for 1hr. Each tube contained 100ml each of test compound, iodinated ligand (7 fmol) and rat membrane (16 mg) added in this order. The assay buffer contained 20mM HEPES pH7.2, 75 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% BSA and protease inhibitors, 2 mM leupeptin and 0.5U aprotinin. The  
30 nonspecific binding was determined in the presence of either 17nM GVIA or 100 nM MVIIC. Assays were terminated by vacuum filtration on a Millipore manifold filtration

- 27 -

system using glass fibre filters (Whatman GFB) presoaked in 0.6% polyethylenimine. Each tube was washed 3 times with 3ml ice-cold wash buffer (20mM HEPES pH7.2, 125mM NaCl and 0.1% BSA). Filters were counted on a gamma counter. Graphpad Prism was used to generate binding curves and calculate EC<sub>50</sub> values. Values for some of the compounds of the present invention are shown in Table 2.

**Table 2:EC<sub>50</sub> in rat brain binding assays**

PEPTIDE	<sup>125</sup> I GVIA Assay	<sup>125</sup> I MVIIIC Assay
CVID	2.3e <sup>-10</sup>	6.4e <sup>-5</sup>
R <sup>10</sup> -CVID	5.3e <sup>-11</sup>	1.2e <sup>-3</sup>
D <sup>9</sup> R <sup>10</sup> -CVID	7.6e <sup>-10</sup>	<1.0e <sup>-3</sup>

### References

Ahmad, S.N., *et al.*, (1988) *Brain Res.*, **453**, 247-56.

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5 Sarin V.K. *et al.*, *Anal.Biochem* **117**, 147 (1981).

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- 29 -

## SEQUENCE LISTING

### (1) GENERAL INFORMATION:

5

(i) APPLICANT: TO BE DECIDED

(ii) TITLE OF INVENTION: NOVEL PEPTIDES

(iii) NUMBER OF SEQUENCES: 13

10

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE

(B) STREET: 1 LITTLE COLLINS STREET

(C) CITY: MELBOURNE

15

(D) STATE: VICTORIA

(E) COUNTRY: AUSTRALIA

(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

20

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: NEW PROV

(B) FILING DATE:

(C) CLASSIFICATION:

30

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: CAINE, MICHAEL J

- 30 -

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5 (B) TELEFAX: +61 3 9254 2770

(C) TELEX: AA 31787

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15 Ser Gly Thr Val Gly Arg  
1 5

20 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

---

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

35 Ser Lys Leu Met Tyr Asp  
1 5

(2) INFORMATION FOR SEQ ID NO:3:

40

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid



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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

10 Ser Arg Leu Met Tyr Asp  
1 5

(2) INFORMATION FOR SEQ ID NO:4:

- 15 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 6 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asp Arg Leu Met Tyr Asp  
1 5

30

(2) INFORMATION FOR SEQ ID NO:5:

- 35 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 27 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: peptide

- 33 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

5 Cys Lys Ser Lys Gly Ala Lys Cys Ser Lys Leu Met Tyr Asp Cys Cys  
 1 5 10 15  
 Ser Gly Ser Cys Ser Gly Thr Val Gly Arg Cys  
 20 25

10

(2) INFORMATION FOR SEQ ID NO:6:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

25 Cys Lys Ser Lys Gly Ala Lys Cys Ser Arg Leu Met Tyr Asp Cys Cys  
 1 5 10 15  
 Ser Gly Ser Cys Ser Gly Thr Val Gly Arg Cys  
 20 25

30

(2) INFORMATION FOR SEQ ID NO:7:

35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

- 34 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

5 Cys Lys Ser Lys Gly Ala Lys Cys Asp Arg Leu Met Tyr Asp Cys Cys  
 1 5 10 15  
 Ser Gly Ser Cys Ser Gly Thr Val Gly Arg Cys  
 20 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 25 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

25 Cys Lys Gly Lys Gly Ala Lys Cys Ser Arg Leu Met Tyr Asp Cys Cys  
 1 5 10 15  
 Thr Gly Ser Cys Arg Ser Gly Lys Cys  
 20 25

30

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 26 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

- 35 -

Cys Lys Gly Lys Gly Ala Pro Cys Arg Lys Thr Met Tyr Asp Cys Cys  
 1 5 10 15  
 Ser Gly Ser Cys Gly Arg Arg Gly Lys Cys  
 5 20 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:  
 10 (A) LENGTH: 27 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

20 Cys Lys Ser Hyp Gly Ser Ser Cys Ser Hyp Thr Ser Tyr Asn Cys Cys Arg Ser  
 1 5 10 15  
 Cys Asn Hyp Tyr Thr Lys Arg Cys Tyr  
 25 20 25

(2) INFORMATION FOR SEQ ID NO:11:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 18 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCGGCACCG TAGGTAGA

- 36 -

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 382 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 15 (B) LOCATION: 10..231

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

20	ATCATCAAA ATG AAA CTG ACG TGT GTG GTG ATC GTC GCC GTG CTG CTC	48
	Met Lys Leu Thr Cys Val Val Ile Val Ala Val Leu Leu	
	1 5 10	
	CTG ACG GCC TGT CAA CTC ATC ACA GCT AAT GAC TCC AGA GGT ACG CAG	96
25	Leu Thr Ala Cys Gln Leu Ile Thr Ala Asn Asp Ser Arg Gly Thr Gln	
	15 20 25	
	AAG CAT CGT GCC CTG AGG TCG GAC ACC AAA CTC TCC ATG TCG ACT CGC	144
	Lys His Arg Ala Leu Arg Ser Asp Thr Lys Leu Ser Met Ser Thr Arg	
30	30 35 40 45	
	TGC AAG AGT AAA GGA GCA AAA TGT TCA AAG CTT ATG TAT GAC TGC TGC	192
	Cys Lys Ser Lys Gly Ala Lys Cys Ser Lys Leu Met Tyr Asp Cys Cys	
	50 55 60	
35	AGC GGT TCT TGC AGC GGC ACC GTA GGT AGA TGT GGC TGATCCGGCG	238
	Ser Gly Ser Cys Ser Gly Thr Val Gly Arg Cys Gly	
	65 70	
40	CTTGATCTCC CCCTTCTGTG CTCTATCCTT TTCTGCCTGA GTCCTCCTTA CCTGAGAGTG	298
	GTCATGAACC ACTCATCACC TACCCCCTGG AGGTCTCAAA GAACTACTTG AAATAAAGCC	358

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GCTTGCAAAA AAAAAAAAAA AAAA

382

(2) INFORMATION FOR SEQ ID NO:13:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

15 Met Lys Leu Thr Cys Val Val Ile Val Ala Val Leu Leu Leu Thr Ala  
 1 5 10 15

Cys Gln Leu Ile Thr Ala Asn Asp Ser Arg Gly Thr Gln Lys His Arg  
 20 25 30

20

Ala Leu Arg Ser Asp Thr Lys Leu Ser Met Ser Thr Arg Cys Lys Ser  
 35 40 45

25 Lys Gly Ala Lys Cys Ser Lys Leu Met Tyr Asp Cys Cys Ser Gly Ser  
 50 55 60

Cys Ser Gly Thr Val Gly Arg Cys Gly  
 65 70

30 Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

35 Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or  
 40 more of said steps or features.

### Figure 1

atcatcaaa														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<u>ATG</u>	<u>AAA</u>	<u>CTG</u>	<u>ACG</u>	<u>TGT</u>	<u>GTG</u>	<u>GTG</u>	<u>ATC</u>	<u>GTC</u>	<u>GCC</u>	<u>GTG</u>	<u>CTG</u>	<u>CTC</u>	<u>CTG</u>	<u>ACG</u>
M	K	L	T	C	V	V	I	V	A	V	L	L	L	T
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
GCC	TGT	CAA	CTC	ATC	ACA	GCT	AAT	GAC	TCC	AGA	GGT	ACG	CAG	AAG
A	C	Q	L	I	T	A	N	D	S	R	G	T	Q	K
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
CAT	CGT	GCC	CTG	AGG	TCG	GAC	ACC	AAA	CTC	TCC	ATG	TCG	ACT	CGC
H	R	A	L	R	S	D	T	K	L	S	M	S	T	R
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
TGC	AAG	AGT	AAA	GGA	GCA	AAA	TGT	TCA	AAG	CTT	ATG	TAT	GAC	TGC
C	K	S	K	G	A	K	C	S	K	L	M	Y	D	C
61	62	63	64	65	66	67	68	69	70	71	72	73	74	tcc
TGC	AGC	GGT	TCT	TGC	AGC	GGC	ACC	GTA	GGT	AGA	TGT	GGC	TGA	tcc
C	S	G	S	C	S	G	T	V	G	R	C	G		

ggcgcttgatctcccccttctgtgctctatccttttctgcctgagtcctccttacctga  
gagtggatcatgaaccactcatcacctacccccctggaggtctcaaagaactacttgaaat  
aaagccgcttgcaaaaaaaaaaaaaaaaaaaaaa

